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A benzthiazole-based tripodal chemosensor for Ba^{2+} recognition under biological conditions

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ABSTRACT

We synthesized a benzthiazole-based chemosensor with mixed S,N donor sites. The binding units are incorporated in a highly flexible tripodal framework which facilitates the complexation of the binding of larger-sized metals. The chemosensor resulted in the sensitive and selective recognition of Ba^{2+} through the enhancement of the fluorescence intensity when studied in a THF/H_2O (8:2, v/v) solvent mixture. The sensor offers an interesting opportunity to monitor Ba^{2+} in biological samples including the cytoplasm of microbes such as *Saccharomyces cerevisiae*.

The development of highly sensitive and reliable methods for the quantification of various cations in the environment is currently of interest.[1] Many research groups have developed selective sensors for alkali and alkaline earth cations. Recent reports concerning the detection of such analytes in biological samples are available.[2] Detection of such metal ions in biological samples is considered necessary in research areas including environmental and life sciences. Among the alkaline earth metal ions, a number of chemosensors are available for Ca^{2+} and Mg^{2+} recognition and even the detection of intracellular Ca^{2+} and Mg^{2+} is commonly performed by employing fluorescent probes.[3] By comparison, only a few examples of selective fluorescent chemosensors for Ba^{2+} have been successfully demonstrated.[4] The development of new selective and sensitive systems for Ba^{2+} estimation are required to monitor potential problems related to its toxicity.[5] The available receptor designs for alkaline earth metal ions are fabricated with crown ethers, carboxylates, and other oxygen and nitrogen donor sites.[6] The main disadvantage of such systems as a receptor of Ba^{2+} is the inherent strong binding affinity of these sites for Ca^{2+} and Mg^{2+}. Therefore, the design of novel and specific chemosensors for Ba^{2+} requires determination of the most selective interactions with cations to allow qualitative and quantitative determination of the parameters of Ba^{2+}. The selectivity demands require obtaining selective metal complexation and fulfilling the steric requirements of barium. The strategy must convert the receptor into the most convenient form for barium complexation with specific physical characteristics.

The design of the receptor is based upon the literature reports of chemosensors demonstrating moderate binding affinity of sp^2 nitrogen donor sites for alkaline earth metal ions.[7] Though the sp^2 nitrogen and thiol groups are usually considered a soft binding site according to HSAB principles, they are generally expected to play a minor role in binding alkali/alkaline earth metal ions.[8] However, some recent reports showed that the inclusion of these sites promotes efficient binding of alkaline earth metal ions.[9] Barium has the largest ionic radius among the alkaline earth metal ions. Thus, this property is judicially used to engineer the receptor. For example, binding sites are incorporated on a larger platform and near the platform in such a way that binding sites may not come together to encapsulate a small sized cation.

Fluorescent chemosensor **2** was synthesized by a reaction of tribromide **1** with 2-thiobenzthiazole by refluxing in acetonitrile in the presence of K_2CO_3 (Scheme 1).[10] Sensor **2** displayed a well-defined band with a λ_{max} of 435 nm in its fluorescence spectrum, which was recorded in a 50 μM THF/H_2O (8:2, v/v) solution excited at a λ_{max} of 380 nm. For the real application of any sensor in biological and environmentally important samples, the sensor must be able to monitor the analyte in water samples. In this context, the sensor was evaluated in a THF/H_2O (8:2, v/v) solvent because the sensor is soluble in THF and this water fraction is sufficient to allow the investigation of samples of biological and environmental

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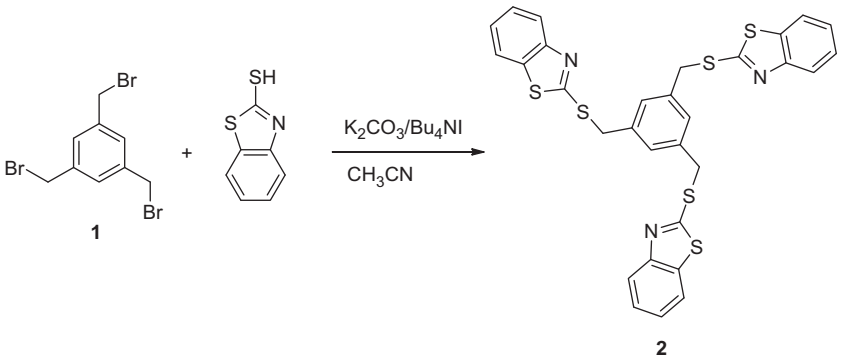

Scheme 1. Synthesis of sensor **2**.

importance. The role of pH on the photophysical properties of sensor **2** was investigated to determine the effect of pH on its emission profile. The variation of pH had no effect on the emission of sensor **2** at λ_{max} = 435 nm, especially in the pH window between 3.9 and 11.5 (Fig. S4).

To investigate the properties of sensor **2** as a potential candidate for metal ion analysis, solutions were prepared in a THF/H_2O solution (8:2, v/v) with a fixed concentration of sensor **2** along with a fixed concentration of a unique metal nitrate salt. The results are shown in Figure 1. A remarkable sensitivity for Ba^{2+} ions can be recognized in Figure 1A and no significant changes were observed with the other tested metal ions. Fe^{3+} influenced the fluorescence intensity differently than Ba^{2+}. While Ba^{2+} caused enhancement of the intensity, Fe^{3+} caused quenching of the intensity. The changes in the fluorescence intensity of sensor **2** upon addition of different metal ions measured at room temperature at 435 nm are shown in Figure 1B. This type of enhancement of the fluorescence intensity upon binding of metal ions is expected due to molecular rigidification, which directly prevents nonradiactive decay. Hence, the fluorescence intensity is increased.[11] In the present investigation, the molecular rigidification seems to contribute more significantly to the fluorescence enhancement because at low pH, sensor **2** did not cause fluorescence enhancement.

To investigate the properties of sensor **2** as a sensor for Ba^{2+} in THF/H_2O (8:2, v/v), a titration was carried out. Figure 2A illustrates the emission profile of sensor **2** with increasing Ba^{2+} ion concentration in the THF/H_2O (8:2, v/v) solution. Upon continuous addition of Ba^{2+} ions into the solution of sensor **2**, the emission of the 50 μM solution of sensor **2** at 435 nm increased monotonically. The association constant, K_a, of sensor **2** for Ba^{2+} was calculated on the basis of the Benesi–Hildebrand plot[12] to be 4.28×10^3 M^{-1}. The stoichiometry of the complex formed was determined by a Job's plot[13] to be 1:1. Sensor **2** can detect a low concentration of Ba^{2+} with a detection limit of 1.0 μM.[14] The effect of pH on the

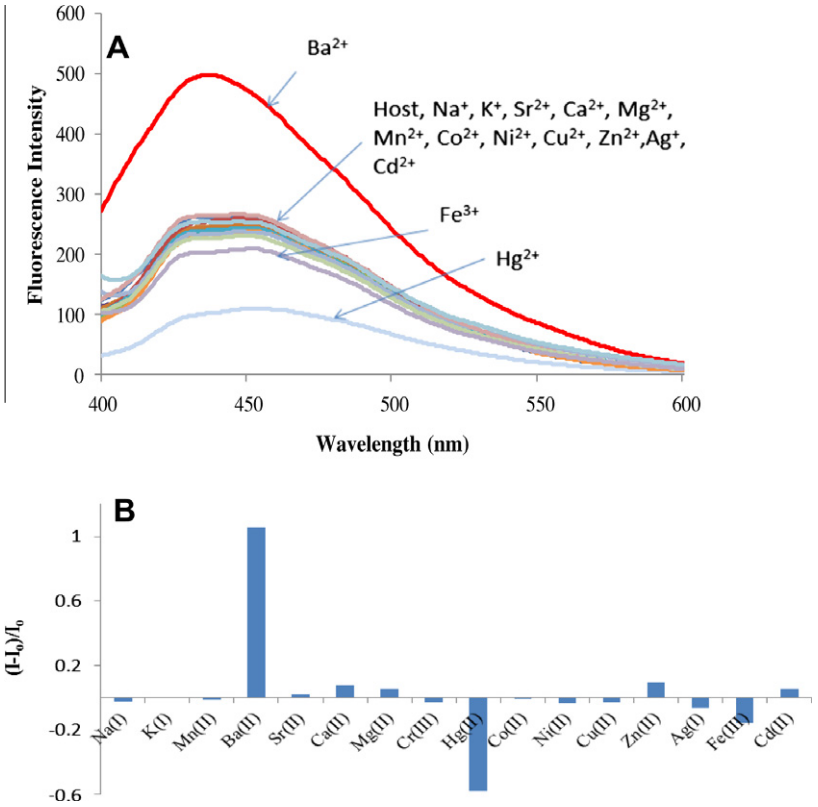

Figure 1. (A) The fluorescence spectra of sensor **2** (50 μM) in the presence of different metal nitrate salts (50 μM) in THF/H_2O (8:2, v/v); (B) Relative intensity ($(I–I_o)/I_o$) of sensor **2** (50 μM) at 435 nm upon addition of different metal nitrate salts (50 μM) in THF/H_2O (8:2, v/v).

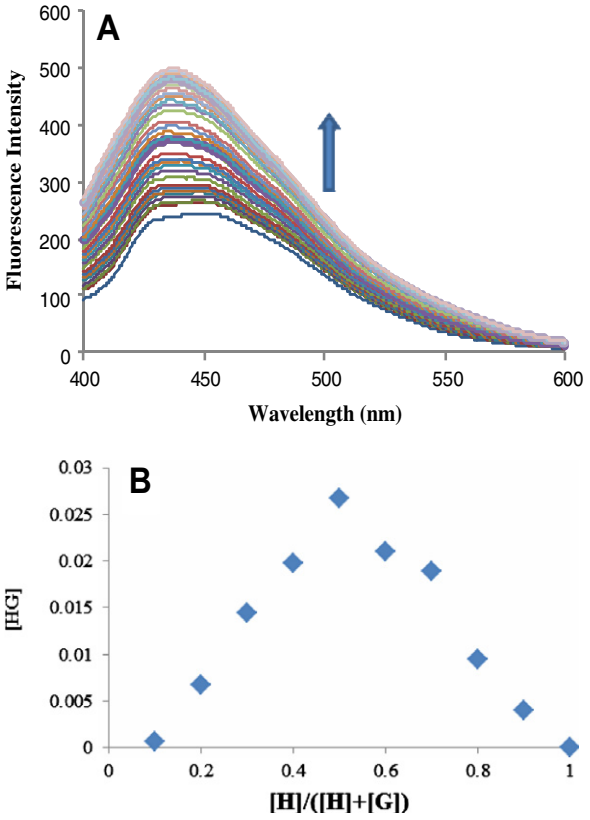

Figure 2. (A) The fluorescence spectra of sensor **2** (50 μM) with increasing concentration of Ba^{2+} ions (0–50 μM) in THF/H_2O (8:2, v/v); (B) Job's plot used to determine the stoichiometry of the complex formed between sensor **2** and Ba^{2+}.

complex of **2** Ba^{2+} was evaluated and it was observed that in the pH range of 3.3 to 11.8, the binding affinity of sensor **2** for Ba^{2+} was not significantly affected (Fig. S6). The effect of the water content in the solution was evaluated to establish the effectiveness of the sensor for the recognition of Ba^{2+} in water samples. It was found that the sensor can detect a concentration range of barium (0–20 μM) in a solvent composition of THF/H_2O (7:3, v/v) (Figure S7). A further increase of the water content retarded the detection range and in THF/H_2O (6:4, v/v), the sensor has a linear relationship for the detection of barium in the range of 0–10 μM (Fig. S8), and a water content of 50% leads to the precipitation of sensor during the course of titration, which results in the broadening of baseline (Fig. S9).

To further confirm the binding behavior, ^{1}H NMR titration was conducted. By comparing the NMR spectra of sensor **2** and **2** Ba^{2+}, it was found that their proton signals differ drastically. Even addition of one equivalent of Ba^{2+} to the solution of sensor **2** caused shifts in all of the signals. Interestingly, the shifts in the singlet of the aromatic platform confirmed that the Ba^{2+} is encapsulated very close to the aromatic platform, thus causing the shifts in the signals. Upon further addition of equivalents, the signals simply overlap the signals of previous spectra except for the emergence of a new signal at 7.47 ppm and this signal was shifted downfield during the course of titration. The emergence of a new signal indicates that with the addition of extra equivalents of Ba^{2+}, asymmetry in the complex appears. However, the lack of a suitable crystal prevents us to comment on the exact structure of the complex. To judge the effect of other metal ions on the signal response of the fluorescence spectrum of sensor **2** and to investigate any possible interference of other metal ions on the barium complexation with sensor **2**, competitive binding experiments were carried out (Fig. 3B). The experiments were performed by measuring the fluorescence intensity at 435 nm in a series of solutions containing sensor **2**, Ba^{2+} ions, and another metal ion added one at a time. The emission at 435 nm was almost identical to that obtained in the absence of any interfering metal ion. These results confirm that the metal ions do not interfere with the signal response induced by Ba^{2+}complexation with sensor **2**.

Several metal ion transporters play a major role in maintaining the correct concentration of various metal ions in different cellular compartments. The *SMF* family of genes is the most pervading one and was found to be related to metal transport in humans.[15] These proteins are homologous in humans and the *Saccharomyces cerevisiae* microbe. In the present investigation, we cultured *Saccharomyces cerevisiae* in normal broth and in experimental media containing Ba^{2+}. The cells cultured in the media containing Ba^{2+} were treated with sensor **2** dissolved in a THF/H_2O (8:2, v/v) solvent mixture. A minimum sensor concentration of 40 μM is required to stain the cells, which may give reasonable fluorescence emission during microscopy. Before performing microscopic observations, the microbe cells were washed with a THF/H_2O (8:2, v/v) solvent mixture. The microscopy images taken of blank microbe cells, microbe cells cultured in a medium enriched with Ba^{2+}, and microbe cells cultured in a medium enriched with Ba^{2+} and treated with sensor **2** are shown in Figure 4. The microscopic investigations revealed that sensor **2** is capable of binding Ba^{2+} in a cellular medium. The microscopic image (Fig. 4C) clearly shows that the sensor passed through the membrane of the microbe and stained the cytoplasm enriched with Ba^{2+}. To confirm that the sensor did not lead to destruction of the microbes, SEM images of microbe cells cultured with Ba^{2+} and treated with sensor **2** were obtained. The smoothness of the microbe surface observed in both cases confirms that the sensor did not cause any damage on the surface of the microbe (Fig. 5).

In conclusion, a new benzthiazole-based chemosensor with a flexible tripodal framework, which facilitated the complexation of Ba^{2+}, was synthesized. The chemosensor was found to provide

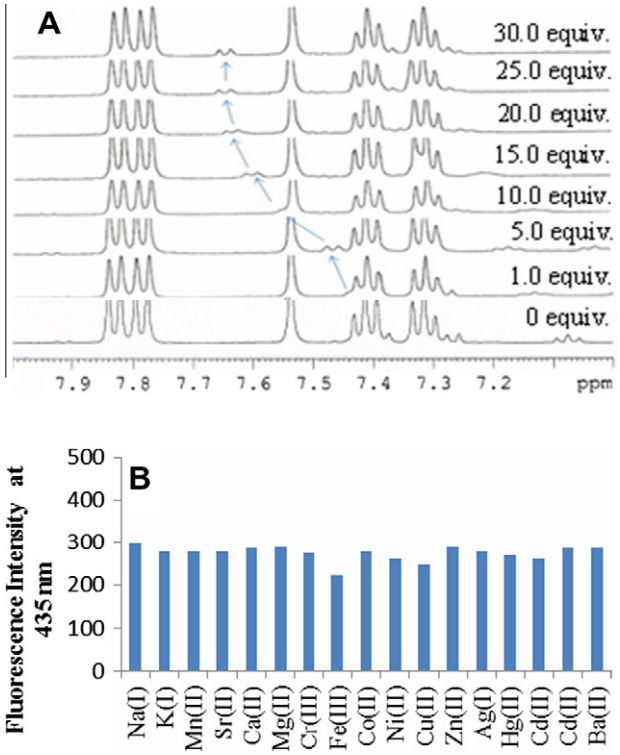

Figure 3. (A) Family of partial ^{1}H NMR spectra of sensor **2** upon successive additions of Ba^{2+}; (B) Estimation of Ba^{2+} in the presence of biologically important metal ions (2 equiv) in the THF/H_2O (8:2, v/v) solvent system where the emission at λ_{max} = 435 nm was used in the calculations.

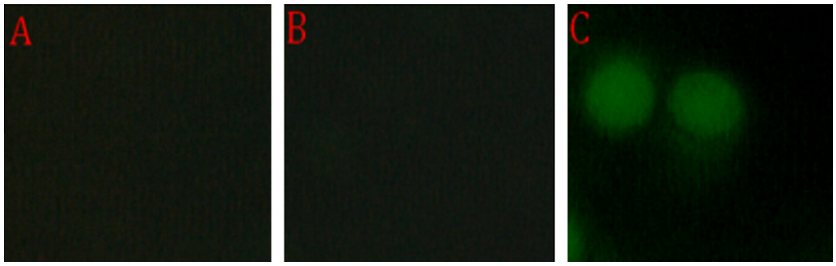

Figure 4. Microscopic images of (A) blank microbe cells; (B) microbe cells cultured in medium enriched with Ba^{2+}, and; (C) microbe cells cultured in medium enriched with Ba^{2+} and treated with sensor **2**. Before performing microscopy, the microbe cells were washed with a THF/H_2O (8:2, v/v) solvent mixture.

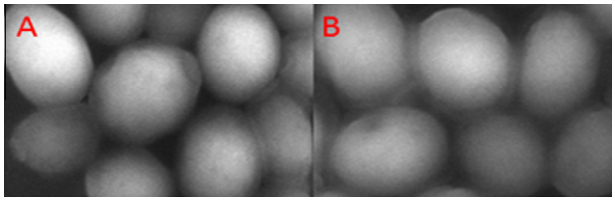

Figure 5. SEM images showing the surface morphology of (A) normal microbe cells and; (B) microbe cells cultured with Ba^{2+} and treated with sensor **2**. Before performing microscopy, the microbe cells were washed with a THF/H_2O (8:2, v/v) solvent mixture.

sensitive and selective recognition of Ba^{2+} through enhancement of the fluorescence intensity. Sensor **2** was tested in various solvent combinations. The optimum results were obtained using a THF/H_2O (8:2, v/v) solvent combination. The sensor offers an interesting opportunity to monitor Ba^{2+} in biological samples such as the cytoplasm of microbes including *S. cerevisiae*. The SEM images demonstrate that sensor **2** did not cause any breakage of the microbe cells.

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Supplementary data

Supplementary data (including spectra and graphs) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2011.09.132.

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10. Synthesis of compound **2**: A solution of tribromide **1** (354 mg, 1.0 mmol), 2-mercaptobenzothiazole (585 mg, 3.5 mmol), and K_2CO_3 (1.38 g, 1.0 mol) along with a catalytic amount of tetrabutylammonium iodide (2 mg) in dry acetonitrile (40 mL) was stirred under reflux for 8 h. Upon completion of the reaction, K_2CO_3 was filtered off and after evaporation of the solvent, the residue was purified by column chromatography on silica gel eluted with hexane/EtOAc (8:2) to yield compound **2** as a white solid (480 mg, 78%). Mp: 105–106 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.52 (s, 6H, $-CH_2$), 7.34 (t, 3H, Ar, J = 8.0 Hz), 7.44 (t, 3H, Ar, J = 8.0 Hz), 7.58 (s, 3H, Ar), 7.83 (d, 3H, Ar, J = 8.0 Hz), 7.91 (d, 3H, Ar, J = 8.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 37.1, 121.2, 121.7, 124.5, 126.0, 129.0, 134.7, 137.3, 152.5, 165.8. Anal. Calcd for $C_{30}H_{21}N_3S_6$: C, 58.50, H, 3.44, N, 6.82; Found C, 58.74; H, 3.30; N, 6.73.
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